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<110> Human Genome Science, Inc.
Principia Pharmaceutical Corporation

<120> Albumin Fusion Proteins

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<141> 2001-04-12

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<170> PatentIn Ver. 2.1

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Ile Ser Ala Asp Ala His Lys Ser
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gaa aat ttc aaa gcc ttg gtg ttg att gcc ttt gct cag tat ctt cag 96
 Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
 20 25 30

cag tgt cca ttt gaa gat cat gta aaa tta gtg aat gaa gta act gaa 144
 Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
 35 40 45

ttt gca aaa aca tgt gtt gct gat gag tca gct gaa aat tgt gac aaa 192
 Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
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tca ctt cat acc ctt ttt gga gac aaa tta tgc aca gtt gca act ctt 240
 Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
 65 70 75 80

cgt gaa acc tat ggt gaa atg gct gac tgc tgt gca aaa caa gaa cct 288
 Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
 85 90 95

gag	aga	aat	gaa	tgc	ttc	ttg	caa	cac	aaa	gat	gac	aac	cca	aac	ctc	336
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ccc	cga	ttg	gtg	aga	cca	gag	gtt	gat	gtg	atg	tgc	act	gct	ttt	cat	384
Pro	Arg	Leu	Val	Arg	Pro	Glu	Val	Asp	Val	Met	Cys	Thr	Ala	Phe	His	
115															125	
gac	aat	gaa	gag	aca	ttt	ttg	aaa	aaa	tac	tta	tat	gaa	att	gcc	aga	432
Asp	Asn	Glu	Glu	Thr	Phe	Leu	Lys	Lys	Tyr	Leu	Tyr	Glu	Ile	Ala	Arg	
130															140	
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Arg	His	Pro	Tyr	Phe	Tyr	Ala	Pro	Glu	Leu	Leu	Phe	Phe	Ala	Lys	Arg	
145															160	
tat	aaa	gct	gct	ttt	aca	gaa	tgt	tgc	caa	gct	gct	gat	aaa	gct	gcc	528
Tyr	Lys	Ala	Ala	Phe	Thr	Glu	Cys	Cys	Gln	Ala	Ala	Asp	Lys	Ala	Ala	
165															175	
tgc	ctg	ttg	cca	aag	ctc	gat	gaa	ctt	cg	gat	gaa	ggg	aag	gct	tcg	576
Cys	Leu	Leu	Pro	Lys	Leu	Asp	Glu	Leu	Arg	Asp	Glu	Gly	Lys	Ala	Ser	
180															190	
tct	gcc	aaa	cag	aga	ctc	aaa	tgt	gcc	agt	ctc	caa	aaa	ttt	gga	gaa	624
Ser	Ala	Lys	Gln	Arg	Leu	Lys	Cys	Ala	Ser	Leu	Gln	Lys	Phe	Gly	Glu	
195															205	
aga	gct	ttc	aaa	gca	tgg	gca	gtg	gct	cgc	ctg	agc	cag	aga	ttt	ccc	672
Arg	Ala	Phe	Lys	Ala	Trp	Ala	Val	Ala	Arg	Leu	Ser	Gln	Arg	Phe	Pro	
210															220	
aaa	gct	gag	ttt	gca	gaa	gtt	tcc	aag	tta	gtg	aca	gat	ctt	acc	aaa	720
Lys	Ala	Glu	Phe	Ala	Glu	Val	Ser	Lys	Leu	Val	Thr	Asp	Leu	Thr	Lys	
225															240	
gtc	cac	acg	gaa	tgc	tgc	cat	gga	gat	ctg	ctt	gaa	tgt	gct	gat	gac	768
Val	His	Thr	Glu	Cys	Cys	His	Gly	Asp	Leu	Leu	Glu	Cys	Ala	Asp	Asp	
245															255	
agg	gcg	gac	ctt	gcc	aag	tat	atc	tgt	gaa	aat	cag	gat	tcg	atc	tcc	816
Arg	Ala	Asp	Leu	Ala	Lys	Tyr	Ile	Cys	Glu	Asn	Gln	Asp	Ser	Ile	Ser	
260															270	
agt	aaa	ctg	aag	gaa	tgc	tgt	gaa	aaa	cct	ctg	ttg	gaa	aaa	tcc	cac	864
Ser	Lys	Leu	Lys	Glu	Cys	Cys	Glu	Lys	Pro	Leu	Leu	Glu	Lys	Ser	His	
275															285	

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Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser	
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tta gct gct gat ttt gtt gaa agt aag gat gtt tgc aaa aac tat gct	960
Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala	
305 310 315 320	
gag gca aag gat gtc ttc ctg ggc atg ttt ttg tat gaa tat gca aga	1008
Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg	
325 330 335	
agg cat cct gat tac tct gtc gtg ctg ctg aga ctt gcc aag aca	1056
Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr	
340 345 350	
tat gaa acc act cta gag aag tgc tgt gcc gct gca gat cct cat gaa	1104
Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Asp Pro His Glu	
355 360 365	
tgc tat gcc aaa gtg ttc gat gaa ttt aaa cct ctt gtg gaa gag cct	1152
Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro	
370 375 380	
cag aat tta atc aaa caa aac tgt gag ctt ttt gag cag ctt gga gag	1200
Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu	
385 390 395 400	
tac aaa ttc cag aat gcg cta tta gtt cgt tac acc aag aaa gta ccc	1248
Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro	
405 410 415	
caa gtg tca act cca act ctt gta gag gtc tca aga aac cta gga aaa	1296
Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys	
420 425 430	
gtg ggc agc aaa tgt tgt aaa cat cct gaa gca aaa aga atg ccc tgt	1344
Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys	
435 440 445	
gca gaa gac tat cta tcc gtg gtc ctg aac cag tta tgt gtg ttg cat	1392
Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His	
450 455 460	
gag aaa acg cca gta agt gac aga gtc aca aaa tgc tgc aca gag tcc	1440
Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser	
465 470 475 480	
ttg gtg aac agg cga cca tgc ttt tca gct ctg gaa gtc gat gaa aca	1488

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485	490	495		
tac gtt ccc aaa gag ttt aat gct gaa aca ttc acc ttc cat gca gat 1536				
Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp				
500	505	510		
ata tgc aca ctt tct gag aag gag aga caa atc aag aaa caa act gca 1584				
Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala				
515	520	525		
ctt gtt gag ctt gtg aaa cac aag ccc aag gca aca aaa gag caa ctg 1632				
Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu				
530	535	540		
aaa gct gtt atg gat gat ttc gca gct ttt gta gag aag tgc tgc aag 1680				
Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys				
545	550	555	560	
gct gac gat aag gag acc tgc ttt gcc gag gag ggt aaa aaa ctt gtt 1728				
Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val				
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Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu				
35	40	45		
Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys				
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Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu				
65	70	75	80	

Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
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Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
100 105 110

Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
115 120 125

Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg
130 135 140

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145 150 155 160

Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
165 170 175

Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
180 185 190

Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu
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Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro
210 215 220

Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
225 230 235 240

Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
245 250 255

Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
260 265 270

Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
275 280 285

Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
290 295 300

Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala
305 310 315 320

Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg
325 330 335

Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr

340

345

350

Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu
 355 360 365

Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro
 370 375 380

Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu
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Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro
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Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys
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Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys
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Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His
 450 455 460

Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser
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Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr
 485 490 495

Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp
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the Therapeutic Protein

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<222> (42)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (43)
<223> n equals a,t,g, or c
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33

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<220>
<221> misc_feature
<222> (44)
<223> n equals a,t,g, or c

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<223> n equals a,t,g, or c

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<223> n equals a,t,g, or c

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<222> (51)
<223> n equals a,t,g, or c

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<221> misc_feature
<222> (52)
<223> n equals a,t,g, or c

<400> 28
ctttaaatcg atgagcaacc tcactttgt gtgcatcnnn nnnnnnnnnn nn
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<210> 29
<211> 24
<212> PRT
<213> Artificial Sequence

<220>
<221> signal
<223> signal peptide of natural human serum albumin protein

<400> 29
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg
20

<210> 30
<211> 114
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> forward primer useful for generation of PC4:HSA
albumin fusion VECTOR

<220>
<221> misc_feature
<222> (5)..(10)
<223> BamHI restriction site

<220>
<221> misc_feature
<222> (11)..(16)
<223> Hind III restriction site

<220>
<221> misc_feature
<222> (17)..(27)
<223> Kozak sequence

<220>
<221> misc_feature
<222> (25)..(97)
<223> cds natural signal sequence of human serum albumin

<220>
<221> misc_feature
<222> (75)..(81)

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<223> XhoI restriction site

<220>
<221> misc_feature
<222> (98)..(114)
<223> cds first six amino acids of human serum albumin

<400> 30
tcagggatcc aagcttccgc caccatgaag tggtaacct ttatccct tcttttctc 60
tttagctcggtt cttactcgag gggtgtgttt cgtcgagatg cacacaagag tgag 114

<210> 31
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> reverse primer useful for generation of
PC4:HSA albumin fusion VECTOR

<220>
<221> misc_feature
<222> (6)..(11)
<223> Asp718 restriction site

<220>
<221> misc_feature
<222> (12)..(17)
<223> EcoRI restriction site

<220>
<221> misc_feature
<222> (15)..(17)
<223> reverse complement of stop codon

<220>
<221> misc_feature
<222> (18)..(25)
<223> AscI restriction site

<220>
<221> misc_feature
<222> (18)..(43)
<223> reverse complement of DNA sequence encoding last 9 amino acids

<400> 31
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gcagcggtac cgaattcggc gcgccttata agcctaaggc agc

43

<210> 32
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> forward primer useful for inserting Therapeutic protein into pC4:HSA vector

<220>
<221> misc_feature
<222> (29)
<223> n equals a,t,g, or c

<220>
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<223> n equals a,t,g, or c

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<222> (31)
<223> n equals a,t,g, or c

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<222> (32)
<223> n equals a,t,g, or c

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<222> (33)
<223> n equals a,t,g, or c

<220>
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<223> n equals a,t,g, or c

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<223> n equals a,t,g, or c

<220>
<221> misc_feature

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<222> (36)
<223> n equals a,t,g, or c

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<222> (37)
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<222> (38)
<223> n equals a,t,g, or c

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<222> (39)
<223> n equals a,t,g, or c

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<220>
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<222> (43)
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<220>
<221> misc_feature
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<221> misc_feature
<222> (45)
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<221> misc_feature
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<400> 32
ccggcgctcg aggggtgtgt ttcgtcgann nnnnnnnnnn nnnnnn 46

<210> 33
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> reverse primer useful for inserting Therapeutic
protein into pC4:HSA vector

<220>
<221> misc_feature
<222> (38)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
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<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (40)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (41)
<223> n equals a,t,g, or c

<220>
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<222> (42)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (43)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
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<222> (44)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (45)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (46)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (47)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (48)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (49)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (50)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (51)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (52)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (53)
<223> n equals a,t,g, or c

<220>
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<221> misc_feature
<222> (54)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (55)
<223> n equals a,t,g, or c

<400> 33
agtcccatcg atgagcaacc tcactttgt gtgcacnnnn nnnnnnnnnn nnnnn      55

<210> 34
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<221> signal
<223> Stanniocalcin signal peptide

<400> 34
Met Leu Gln Asn Ser Ala Val Leu Leu Leu Val Ile Ser Ala Ser
 1           5           10          15

Ala

<210> 35
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<221> signal
<223> Synthetic signal peptide

<400> 35
Met Pro Thr Trp Ala Trp Trp Leu Phe Leu Val Leu Leu Leu Ala Leu
 1           5           10          15

Trp Ala Pro Ala Arg Gly
 20

<210> 36
<211> 733
<212> DNA
<213> Homo sapiens

<400> 36

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gggatccgga	gcccaaatct	tctgacaaaa	ctcacacatg	cccaccgtgc	ccagcacctg	60
aattcgaggg	tgcaccgtca	gtcttcctct	tccccccaaa	acccaaggac	accctcatga	120
tctccggac	tcctgaggtc	acatgcgtgg	tggtggacgt	aagccacgaa	gaccctgagg	180
tcaagttcaa	ctggtaacgtg	gacggcgtgg	aggtgcataa	tgccaagaca	aagccgcggg	240
aggagcagta	caacagcacg	taccgtgtgg	tcagcgtcct	caccgtcctg	caccaggact	300
ggctgaatgg	caaggagtagc	aagtgcagg	tctccaacaa	agccctccca	accccccattcg	360
agaaaaccat	ctccaaagcc	aaagggcagc	cccgagaacc	acaggtgtac	accctgcccc	420
catcccgga	tgagctgacc	aagaaccagg	tcagcctgac	ctgcctggtc	aaaggcttct	480
atccaagcga	catcgccgtg	gagtgggaga	gcaatgggca	gccggagaac	aactacaaga	540
ccacgcctcc	cgtgctggac	tccgacggct	ccttcttcct	ctacagcaag	ctcaccgtgg	600
acaagagcag	gtggcagcag	gggaacgtct	tctcatgctc	cgtgatgcat	gaggctctgc	660
acaaccacta	cacgcagaag	agcctctccc	tgtctccggg	taaatgagtg	cgacggccgc	720
gactctagag	gat					733

<210> 37
 <211> 5
 <212> PRT
 <213> Artificial sequence

<220>
 <221> misc_structure
 <223> membrane proximal motif of class 1 cytokine receptors

<220>
 <221> misc_feature
 <222> (3)
 <223> Xaa equals any

<400> 37
 Trp Ser Xaa Trp Ser
 1 5

<210> 38
 <211> 86
 <212> DNA

<213> Artificial Sequence

<220>

<221> primer_bind

<223> forward primer useful for generation of a synthetic gamma activation site (GAS) containing promoter element

<400> 38

gcgcctcgag atttccccga aatcttagatt tccccgaaat gatttccccg aatgatttc 60

cccgaaatat ctgccatctc aattag

86

<210> 39

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<221> primer_bind

<223> reverse primer useful for generation of a synthetic gamma activation site (GAS) containing promoter element

<400> 39

gcggcaagct ttttgcaaag ccttaggc 27

<210> 40

<211> 271

<212> DNA

<213> Artificial Sequence

<220>

<221> misc_feature

<223> Synthetic GAS-SV40 promoter sequence

<400> 40

ctcgagattt ccccgaaatc tagatttccc cgaaatgatt tccccgaaat gatttccccg 60

aaatatctgc catctcaatt agtcagcaac catagtcccg cccctaactc cgcccatccc 120

gcccttaact ccgcccagtt ccgcccattc tccgccccat ggctgactaa ttttttttat 180

ttatgcagag gccgaggccg cctcggcctc tgagctattc cagaagttagt gaggaggctt 240

ttttggaggc ctaggctttt gcaaaaagct t 271

<210> 41

<211> 32

<212> DNA

<213> Artificial Sequence

<220>
<221> primer_bind
<223> primer useful for generation of a EGR/SEAP reporter construct

<400> 41
gcgctcgagg gatgacagcg atagaacccc gg 32

<210> 42
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> primer useful for generation of a EGR/SEAP reporter construct

<400> 42
gcgaagcttc gcgactcccc ggatccgcct c 31

<210> 43
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_binding
<223> NF-KB binding site

<400> 43
ggggactttc cc 12

<210> 44
<211> 73
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> forward primer useful for generation of a vector containing the NF-KB promoter element

<400> 44
gcggcctcga ggggactttc ccggggactt tccggggact ttccgggact ttccatcctg 60
ccatctcaat tag 73

<210> 45

<211> 256
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> Synthetic NF-KB/SV40 promoter

<400> 45
ctcgagggga ctttcccgaa gactttccgg ggactttccg ggactttcca tctgccatct 60
caatttagtca gcaaccatag tccccccctt aactccgccc atcccgcccc taactccgccc 120
cagttccgccc cattctccgc cccatggctg actaattttt tttattttatg cagaggccgaa 180
ggccgcctcg gcctctgagc tattccagaa gtatgtgagga ggctttttg gaggcctagg 240
cttttgcaaa aagctt 256